AMENDMENTS TO THE CLAIMS:

Please amend claims 1, 2, 7, 16, 45, 53, 54, 57, 59, 61, 63, 64 and 65, and cancel claims 46 and 47 without prejudice or disclaimer. This listing of claims replaces all prior versions, and listings of claims, in the application.

LISTING OF CLAIMS:

1. (Currently amended) A method of producing and identifying a mammalian protease mutein ~~that cleaves~~ with increased cleavage activity and/or altered substrate specificity for ~~a substrate sequence in~~ a target protein involved with a pathology in a mammal, wherein:

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis;

cleavage of ~~said~~ a substrate sequence in said target protein serves as a treatment for said pathology; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library of protease muteins of a scaffold, wherein:

each different mutein protease in the library ~~being~~ is a member of the library;~~,~~

each member of the library has ~~having~~ N mutations relative to a wild-type mammalian protease scaffold; and ~~wherein~~

N is a positive integer;

(b) measuring [[an]] a cleavage activity and/or substrate specificity of at least two members of the library ~~in cleaving~~ for the substrate sequence; and

(c) identifying at least one mutein protease having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence in the target protein, relative to the wild-type mammalian protease scaffold.

2. (Currently amended) The method of claim 1, wherein the wild-type mammalian protease scaffold is a serine or cysteine protease.

3. (Previously presented) The method of claim 1, wherein N is an integer between 1 and 20.

4. (Previously presented) The method of claim 3, wherein N is an integer from 1-5.

5. (Previously presented) The method of claim 3, wherein N is an integer from 5-10.

6. (Previously presented) The method of claim 3, wherein N is an integer from 10-20.

7. (Currently amended) The method of claim 1, wherein the wild-type mammalian protease scaffold ~~has an amino acid sequence derived from one of the proteases~~ is selected from among trypsin, chymotrypsin, subtilisin, MTSP-1, granzyme A, granzyme B, and granzyme M, elastase, chymase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin and cruzain.

8. (Cancelled)

9. (Previously presented) The method of claim 1, wherein the pathology is selected from among rheumatoid arthritis, sepsis, cancer, acquired immunodeficiency syndrome, respiratory tract infections, influenza, cardiovascular disease and asthma.

10. (Canceled)

11. (Original) The method of claim 1, wherein the target protein is involved in apoptosis.

12. (Previously presented) The method of claim 11, wherein the target protein is caspase-3, VEGF or VEGF-R.

13. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 10-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

14. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 100-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

15. (Previously presented) The method of claim 1, wherein the specificity of the identified protease mutein for cleaving the substrate sequence is increased by at least 1000-fold compared to the specificity of the wild-type mammalian protease scaffold for cleaving the substrate sequence.

16. (Currently amended) The method of claim 1, further comprising the steps of:

(d) ~~providing~~ identifying the mutation(s) contained in a first mutein protease and a second mutein protease ~~two or more members of the protease library~~ identified in step c) ~~with~~ as having increased cleavage activity and/or altered specificity;

(e) producing a third mutein protease containing ~~combining~~ the mutations ~~on a~~ of the first mutein protease ~~with~~ and the mutations ~~on a~~ of the second mutein protease ~~to produce a third mutein protease~~; and

(f) measuring the cleavage activity and/or substrate specificity of the third mutein protease to determine ~~identifying~~ whether ~~the combination produces a combined specificity protease that has~~ its ~~increased~~ cleavage activity and/or ~~altered~~ specificity for the substrate sequence compared to the first mutein protease or second mutein protease.

17-44. (Canceled)

45. (Currently amended) The method of claim 1, ~~wherein the~~ further comprising:

repeating steps a)-c) ~~are repeated~~ iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

~~to create a variant~~ identifying among the further library, a mutein protease having ~~desired~~ altered specificity [[,]] and/or increased cleavage activity relative to the wild-type mammalian protease~~and selectivity~~.

46. (Cancelled)

47. (Cancelled)

48. (Previously presented) The method of claim 1, wherein the substrate sequence is a sequence in a human protein.

49. (Canceled)

50. (Previously presented) The method of claim 1, further comprising the steps of:

providing at least one mutein protease identified in step (c); and

testing the mutein protease in a cell-based assay against a target protein comprising the substrate sequence.

51. (Previously presented) The method of claim 50, wherein the member of the library identified in step (c) has the highest measured cleavage activity.

52. (Previously presented) The method of claim 1, further comprising the steps of providing at least one mutein protease identified in step (c), and testing the mutein protease in an *in vivo* assay.

53. (Currently amended) A method of producing and identifying a mammalian protease mutein ~~that cleaves a substrate sequence in~~ with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a mammal, wherein:

cleavage of ~~said~~ a sequence in said target protein serves as a treatment for said pathology;

the mammalian protease is selected from among granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin and cruzain; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library of protease muteins each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type mammalian protease scaffold, wherein N is a positive integer;

(b) measuring [[an]] a cleavage activity and/or substrate specificity of at least two members of the library ~~in cleaving~~ for the substrate sequence;

(c) identifying at least one protease mutein having ~~a measured increase in~~ increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence relative to the wild-type mammalian protease scaffold;

(d) ~~providing two or more members of the protease mutein library~~ identifying the mutation(s) contained in a first mutein protease and a second mutein protease identified in step c) ~~with~~ as having increased cleavage activity and/or altered specificity for cleaving said substrate sequence;

(e) ~~combining~~ generating a third mutein protease containing the mutations ~~in a~~ of the first mutein protease ~~with increased cleavage activity with~~ and the mutations ~~in a~~ of the second mutein protease ~~to produce a third mutein~~; and

(f) measuring the cleavage activity and/or substrate specificity of the third mutein to determine ~~identifying~~ whether the third mutein produces a protease that has increased cleavage activity toward the substrate sequence and/or altered specificity for cleaving said substrate sequence compared to the first mutein protease or second mutein protease.

54. (Currently amended) The method of claim 53, ~~the method~~ further comprising the steps of:

(g) providing at least one protease mutein identified in step (c); and

(h) testing the protease mutein in a cell-based assay against a target protein comprising the substrate sequence.

55. (Cancelled)

56. (Previously presented) The method of claim 54, wherein the cell-based assay is an *in vivo* assay.

57. (Currently amended) The method of claim 53, ~~wherein~~ further comprising:

repeating ~~the~~ steps a)-c) ~~are repeated~~ iteratively to produce a further library of protease muteins from the identified muteins each with increased cleavage activity and/or altered substrate specificity; and

~~to create a variant~~ identifying among the further library, a mutein protease having ~~desired~~ altered specificity [[,]] and/or increased cleavage activity relative to the wild-type mammalian protease~~and selectivity~~.

58. (Previously presented) The method of claim 53, wherein the substrate sequence is a sequence in a human protein.

59. (Currently Amended) A method of producing and identifying a human protease mutein ~~that cleaves a substrate sequence in~~ with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a human, wherein:

cleavage of ~~said~~ a sequence in said target protein serves as a treatment for said pathology;

the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis; and

the method ~~comprising~~ comprises the steps of:

(a) producing a library of protease muteins, each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type ~~mammalian~~ human protease scaffold, wherein:

N is a positive integer; and[[,]] ~~wherein said~~

the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M, cathepsin, trypsin, chymotrypsin, subtilisin, MTSP-1, elastase, chymase, tryptase, collagenase, papain, neutrophil elastase, complement factor serine proteases, ADAMTS13, neural endopeptidase/neprilysin, furin and cruzain;

(b) measuring [[an]] a cleavage activity and/or substrate specificity of at least two members of the library ~~in cleaving~~ for the substrate sequence~~, wherein the target protein is selected from among a cell surface molecule that transmits an extracellular signal for cell~~

~~proliferation, a cytokine, a cytokine receptor and a signaling protein that regulates apoptosis~~; and

(c) identifying at least one protease mutein having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence, relative to the wild-type ~~mammalian~~ human protease scaffold.

60. (Previously presented) The method of claim 59, wherein step (c) is accomplished by identifying at least one protease mutein having altered substrate specificity for cleaving said substrate sequence, relative to the wild-type scaffold sequence.

61. (Currently Amended) The method of claim 59, wherein the wild-type protease scaffold is Granzyme B or MTSP-1.

62. (Previously presented) The method of claim 59, wherein the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2) and cyclin dependent kinase-4 (cdk-4).

63. (Currently amended) A method of producing and identifying a human protease mutein ~~that cleaves a substrate sequence in~~ with increased cleavage activity and/or altered substrate specificity for a target protein involved with a pathology in a mammal, wherein:

cleavage of ~~said~~ a sequence in said target protein serves as a treatment for said pathology;

the target protein is selected from among caspase 3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41,

hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4); and

the method ~~comprising~~ comprises the steps of:

(a) producing a library of human protease muteins, each different protease mutein in the library being a member of the library, each member having N mutations relative to a wild-type human protease scaffold ~~sequence of a human protease~~ wherein:

N is a positive integer; and [[,]] ~~wherein said~~

the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M, cathepsin, MTSP-1, elastase, chymase, tryptase, chymotrypsin, collagenase, factor Xa, Protein C, plasma kallikrein, plasmin, trypsin, thrombin, complement factor serine proteases, papain, ADAMTS13, endopeptidase, furin, cruzain and plasminogen activator;

(b) measuring [[an]] a cleavage activity and/or substrate specificity of at least two members of the library ~~in cleaving~~ for the substrate sequence~~, wherein said target protein is selected from among caspase-3, tumor necrosis factor, tumor necrosis factor receptor, interleukin-1, interleukin-1 receptor, interleukin-2, interleukin-2 receptor, interleukin-4, interleukin-4 receptor, interleukin-5, interleukin-5 receptor, interleukin-12, interleukin-12 receptor, interleukin-13, interleukin-13 receptor, p-selectin, p-selectin glycoprotein ligand, Substance P, Bradykinin, PSGL, factor IX, immunoglobulin E, immunoglobulin E receptor, CCR5, CXCR4, glycoprotein 120, glycoprotein 41, hemagglutinin, respiratory syncytium virus fusion protein, B7, CD28, CD2, CD3, CD4, CD40, vascular endothelial growth factor, VEGF receptor, fibroblast growth factor, endothelial growth factor, EGF receptor, TGF receptor, transforming growth factor, Her2, CCR1, CXCR3, CCR2, Src, Akt, Bcl-2, BCR-Abl, glucagon synthase kinase-3, cyclin dependent kinase-2 (cdk-2), and cyclin dependent kinase-4 (cdk-4)~~; and

(c) identifying at least one protease mutein having an increased cleavage activity and/or altered substrate specificity for cleaving said substrate sequence, relative to the wild-type ~~mammalian~~ human protease scaffold.

64. (Currently Amended) The method of claim 63, wherein step (c) is accomplished by identifying at least one protease mutein having altered substrate specificity

for cleaving said substrate sequence, relative to the wild-type ~~mammalian~~ human protease scaffold.

65. (Currently amended) The method of claim 63, wherein the human protease scaffold is selected from among granzyme A, granzyme B, granzyme M and MTSP-1.

66. (Previously presented) The method of any one of claims 63-65, wherein the target protein is selected from among caspase 3, vascular endothelial growth factor and VEGF receptor.